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BIOLOGICAL CONVERSION OF ANIMAL WASTES TO NUTRIENTS

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

In an attempt to solve the environmental problem of manure disposal, the study described here employed the larvae of house flies to process poultry manure to produce a feed supplement for baby chicks. Feeding trials that utilized fly pupae and catabolized poultry manure as protein sources for growing chickens were evaluated. The report should be of interest not only to persons concerned with the disposal of solid waste but also to those in the poultry industry.

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ABSTRACT

BIOLOGICAL CONVERSION OF ANIMAL WASTES TO NUTRIENTS

Fly eggs were placed in fresh poultry manure under different temperature (22° to 38° C.) and relative humidity (19 to 80%) conditions to determine the hatchability of fly eggs, growth of larvae, production of pupae and physical changes in the poultry manure. Moisture loss from the manure was recorded daily. The inoculation rates varied from 2 g. to 5 g. fly eggs per 4000 g. of fresh poultry manure. Fly eggs and larvae were also placed under caged laying hens for the catabolism of the manure as it was voided.

Optimum yield of dry pupae was obtained with a combination of 3 g. fly eggs in 4000 g. of fresh poultry manure at 27° C. and a relative humidity of 41%. Moisture in the manure was reduced from 78.5 to 55.0%. When relative humidity was increased from 38 to 70% the yield of pupae at 34° to 38°C. was significantly ($P < 0.01$) increased. The combination of 2 g. eggs per 4000 g. of fresh manure produced significantly ($P < 0.01$) heavier larvae.

Hatchability of fly eggs in fresh poultry manure varied from 50.5 to 87.0%. In comparison to fresh poultry manure, the manure residue had less odor, contained less moisture and was granular in texture. The fly larvae developed well under the caged layers,

reducing freshly voided manure to granular material within a few hours.

Three feeding trials were conducted to evaluate fly pupae and catabolized poultry manure residue as protein sources for growing chickens. In the first trial ground dry pupae or manure residue was used to replace soybean meal in a chick starter diet. Single Comb White Leghorn chicks were fed these diets from one day through four weeks of age. No significant ($P < 0.05$) difference in weight gain was obtained between different treatments. Feed conversion of the chicks fed digested poultry manure was significantly ($P < 0.05$) less desirable than the controls.

In the second trial, White Plymouth Rock chicks were fed a diet containing dried ground pupae as the only source of protein supplement from one day to seven weeks of age. These chicks received no trace mineral or B-vitamin supplements in their diet. In comparison to control chicks receiving a fully balanced diet, no significant ($P < 0.05$) differences in body weight or feed conversion were found.

In the third trial, both fish meal, and meat and bone meal were replaced with fly pupae in the broiler diet. Digested manure was used to replace milo at 5 and 10% levels. New Hampshire and Indian River broiler chicks were fed these diets from one day through

four weeks of age. No significant differences ($P < 0.05$) in weight gain and feed conversion were found between different treatments.

The metabolizable energy of the fly pupae and digested poultry manure was determined to be 2528 Cal/kg. and 580 Cal/kg. respectively.

CHAPTER I

INTRODUCTION

The poultry industry is vitally concerned with the problem of manure disposal. It continuously accentuates as production units are enlarged in size and the concentration of number of birds per unit area is increased. As a result, very few poultrymen can continue to follow the practice of disposal of this product as fertilizer. Although manure is a balanced source of nutrients which can improve the soil structure when used as a fertilizer the value of these properties do not exceed the cost of hauling and spreading. The disposal of poultry manure by the plow-furrow-cover technique appears to be an excellent conservation method, but is quite expensive and laborious. The use of lagoons is limited to operations where large quantities of inexpensive water are available. Automated handling of poultry waste in cage operations has been attempted for many years, but high capital outlay and equipment depreciation costs tend to limit wide application. In addition to handling difficulties it is becoming increasingly expensive to transport large quantities of used litter or droppings from the poultry house to a dumping site. It is often difficult to find suitable disposal sites for large quantities of poultry manure throughout the year.

Ideally poultry wastes should be utilized economically, in a fashion which prevents the spread of disease and pollution of air, soil and water. If possible, wastes should be recycled to avoid an unnecessary drain on our resources. From the existing evidence, it appears that no one method of poultry manure disposal has universal application.

Disposal of any material requires that the physical and/or chemical properties be altered so that the material can be utilized, dumped or destroyed without becoming a nuisance or a health hazard. Biodegradation is the use of natural living organisms to catabolize the poultry manure that may provide a practical means of solving the difficulty. These studies were conducted at Colorado State University Avian Science Research Center to determine how living organisms may be used to catabolize poultry manure. The larvae of house flies (*Musca domestica*) were used to process poultry manure for the production of a fertilizer or soil conditioner and as a feed supplement.

CHAPTER II

REVIEW OF LITERATURE

There are only a few reports available in the literature on the biodegradation of poultry manure with Musca domestica. The idea of utilizing a coprophagous insect, specifically the house fly, as a potential means of handling waste products is not new. It was first suggested by a German researcher, Linder (1919). He proposed that house fly larvae could be used to consume human wastes which could in turn be harvested as a potential protein source.

Recently Calvert, et al. (1969) reported that dried fly pupae provided enough protein of suitable quality to support normal growth of baby chicks during the first two weeks of life. They further reported that the house fly could be used to convert waste material into usable high quality nutrient supplements. Such use would be one way of alleviating the problem of organic wastes that is so large a part of the general problem of pollution.

Miller (1969) conducted a preliminary investigation in which fly pupae were compared to other feedstuffs. When fed in a chick starter ration, the results indicated that fly pupae may be an adequate source of protein for chickens. Amino acid analysis indicated that fly pupae are comparable to fish meal as a protein supplement.

Feeding value of Assam Muga Silkworm pupae for chicks was reported by Bora and Sharma (1965). They found that Assam Muga Silkworm pupae used as an animal protein supplement in chick mash is as good as fish meal. They further suggested an unknown growth factor in Assam Muga Silkworm pupae.

Poultry manure is generally higher in plant nutrients, particularly nitrogen, than other farm manures. The feces generally contains roughly one-half of the nitrogen, nearly all of the phosphorus, and one-third of the potassium excreted (Salter and Schollenberger, 1939). Breon (1963) at the University of New Hampshire found that urinary nitrogen varied from 68 to 75%, with the remainder being fecal nitrogen.

Poultry manure contains numerous other plant nutrients in addition to nitrogen, phosphorus, and potassium. Among these are boron, calcium, copper, iron, magnesium, manganese, molybdenum, sulfur and zinc (Benne et al. 1961).

Changes in the microbial and chemical composition of batch lots of dry and liquid poultry manure during decomposition were studied by William et al. (1969). Their careful observations revealed that these changes were related to the production of offensive odors. The decomposition of uric acid by both aerobic and anaerobic uricolytic bacteria appeared to be related to the formation of significant quantities of ammonia. The number of

sulfate reducing bacteria, including desulfovibrio species, is generally increased during the course of decomposition of liquid poultry manure. These organisms are implicated as producers of some of the hydrogen sulfide in liquid poultry waste.

Manure becomes "sour" when micro-organisms attack and metabolize the fecal matter and convert higher order organic compounds into organic volatile acids such as acetic, propionic and other fatty acids (Moore et al. 1950). The volatile acid content of poultry manure may vary from 0.5 to 7.0% of the total solids depending upon the age, amount and kind of biological attack it has undergone. Fatty acids such as butyric, valeric, caproic and caprylic are responsible for the disagreeable odor of manure (Degering, 1957). Also contributing to the odor are diamines which are degradation products from the metabolism of proteins. Mercaptans and skatols may be found in small amounts contributing to the offensive odor. The exact formulation and concentrations of these compounds in poultry manure have not been determined.

Agronomic Value of Poultry Manure:

Poultry manure benefits crop yields in two ways.

1. Directly, through its nutrients content, and
2. Indirectly, by improving soil structure through its organic matter content.

Numerous investigations have shown that poultry manure increases crop production. Swan (1951), in Connecticut reported that a mixture of dropping board and litter manure could be used to supply one-half of the required plant nutrients for tobacco, with the other half being supplied by commercial fertilizer. Four year studies in Delaware by Rohl (1949), demonstrated the value of broiler-house manure on tomatoes, cantaloupes, watermelons and lima beans. Kroontje et al. (1958) in Virginia found that poultry manure litter supplemented with phosphorus and potassium was just as effective in increasing yields of corn as was an equivalent amount of nutrients from commercial fertilizer irrespective of soil type, season and source of manure.

Recently Miller et al. (1969) reported that poultry manure is beneficial for the correction of zinc and iron deficiencies in soil. This benefit is supplementary to its value as nitrogen, phosphorus and potash fertilizer. Furthermore, the organic fraction of poultry manure is important in rendering zinc and iron more available to plants. Their findings support the hypothesis that manure and other organic wastes, may either supply or give rise to natural chelating agents that aid in the solubilization of insoluble micronutrient elements in soil and thereby render them more available to plants.

Poultry manure increased crop yield, but its fertilizer value is low and handling costs for needed amounts of crop nutrients are higher than for comparable chemical fertilizer. Engineering

studies have shown that approximately 1.2 man hours are required to remove and load a ton of poultry manure into a spreader (Klies and Waint, 1960). The same study showed the average overhead cost in 1957, for a manure spreader was 40 cents per hour used. The time required for spreading was not shown and must be added. If the poultry manure is to be applied only to soils of good tilth, its plant nutrients would be its only value. No economic evaluation of poultry manure as a fertilizer was reported in the literature.

Benne et al. (1961) calculated the nutrient value of dairy cattle manure as three times the cost of hauling and handling, that of steer manure was over four times similar costs. Van Arsdall (1962) at Illinois studied the problem of whether it was more economical to buy chemical fertilizers and dispose of liquid hog manure in a lagoon, or spread the hog manure on croplands. The conclusion was that "the most profitable practice for the average farmer who raises hogs in confinement is to dispose of the liquid manure in a lagoon and use commercial fertilizers in his fields." Objections to applying his findings to poultry manure may be raised. Poultry manure is usually reported to be about three times higher in nutrients than hog manure (Salter & Schollenberger, 1939).

Manure Disposal:

A considerable research effort is being focused on the evaluation of manure disposal techniques. Anaerobic lagoons offer

a considerable potential as a method of treating poultry waste. Hart and Turner (1965) have conducted a number of experiments using anaerobic lagoons for poultry, swine, and cattle manure. They have pointed out the objective of such treatment is the destruction and stabilization of organic matter rather than water purification. As such, anaerobic lagoons are more analogous to anaerobic digesters than they are to aerobic oxidation ponds.

Disposal of manure into lagoons has been found the cheapest method of handling hog manure under mid-west conditions (Kesler, 1966). The earthen lagoon with treated wood retaining walls is probably the most economical where seepage can be controlled by clay soil (Adams, 1962). Where conditions are favorable and where proper management is practiced, a lagoon can be a satisfactory means of manure disposal (Eby, 1963).

Treatment methods such as aerobic and anaerobic lagooning (Eby, 1963, 1964; Taiganides, 1964, 1968) and anaerobic digestion (Hart, 1963; Taiganides, 1963; Anthonisen, 1965) have been investigated. All of these treatment techniques require the eventual final disposal of residual solids remaining after treatment (ultimate disposal). Incineration (Ludington and Sobel, 1964) can be used as a technique for ultimate disposal, but it is expensive and may lead to air pollution.

Composting (Eby, 1964; Wiley, 1963) has been used in the past for poultry manure disposal, but it is not profitable. Wiley (1963)

reported that one might not expect a profit by composting, but on the other hand this procedure appears to be the most economically feasible method of treatment and utilization of poultry manure.

Dehydration is one method of changing the physical properties of poultry manure so that it is no longer offensive and still retains most of its original fertilizer value. The problem of marketing large quantities of dehydrated product has created the greatest concern. If this obstacle can be overcome, the practice of dehydration could be very satisfactory (Ostrander, 1969).

Hydraulic collection of poultry waste utilizes a pit of varying depth and width with or without water added initially. Ludington and Sobel (1964) reported the greatest advantage of the hydraulic system is flexibility in the cleaning schedule. The hydraulic system is often referred to as inside lagoons (Ostrander, 1964). These are not lagoons in the true sense of the word. Lagoons depend on photosynthetic (solar) action and aeration to encourage algae growth. There is no photosynthesis in these inside tanks, as the direct rays of the sun cannot get to them.

The disposal of liquid manure by the plow-furrow-cover technique appears to be an excellent conservation method. With this method from 1 to 2 inches of slurry is deposited in a plowed furrow 6 to 8 inches deep. Immediately after deposition, a single bottom plow covers the manure while making the next furrow.

Depositing and completely covering two inches of slurry, equivalent to 225 tons per acre, has been very successful (Reed, 1966, 1969).

No data were presented on the economic value of this method.

CHAPTER III

METHODS AND MATERIALS

Fly Cages:

Three fly cages (Figure 1) each 232 cm. long, 245 cm. high and 61 cm. deep, were used for the breeder stock of Musca domestica. Each cage had four access holes, 20 cm. in diameter, through which the flies were fed, watered and eggs collected. A metal flange was formed around the hole to extend outward approximately 10 cm. and inward 2.5 cm.. A cotton sleeve approximately 45 cm. long was placed over the outer flange and held in place by rubber bands. The sleeve closed the opening and aided in keeping the flies in the cage.

Feeding:

The breeder flies were fed a dry diet consisting of 454 g. dried milk, 100 g. dried yeast and 20 g. granulated sugar. All ingredients were thoroughly mixed and a thin layer of approximately 3 mm. was spread in the bottom of a shallow plastic container (Figure 2). The diameter and height of the container were 12.5 cm. and 4 cm., respectively.

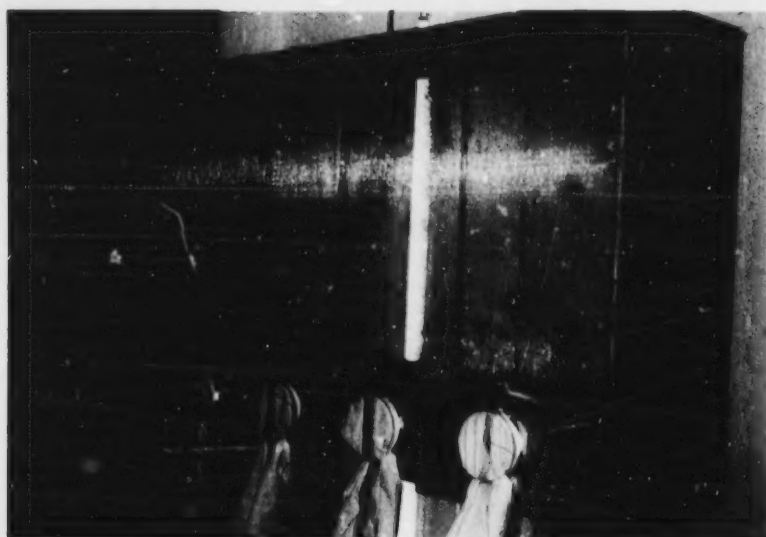


Figure 1. Cages used for confining adult breeder flies (*Musca domestica*).

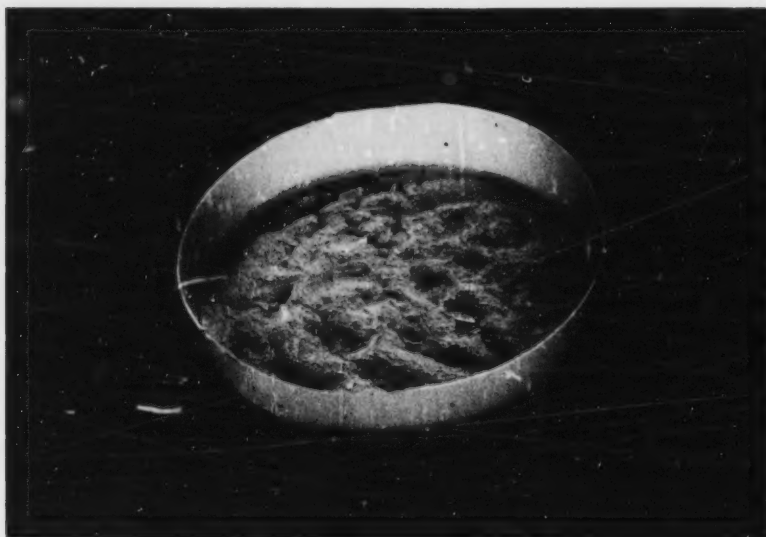


Figure 2. Feeding tray containing feed used to nourish adult breeder flies.

Watering:

Water (Figure 3) was provided by filling a plastic beaker (height, 11 cm., diameter 8.5 cm. and capacity 400 ml.) and inverting it into a plastic dish (diameter 12.5 and height 4 cm.). Paper towels were put in between the shallow plastic container and beaker. The moist surface of the paper towel served as an excellent watering surface for the adult flies.

Egg Deposition and Collection:

Two shallow plastic containers were used for the egg deposition (diameter 9 cm. and height 3 cm.). One container was filled with approximately 80 g. of fresh poultry manure. The second container was inverted over the other, leaving a small crack for adult flies to enter and deposit their eggs (Figure 4). Eggs were separated from the manure with the help of a small brush and blunt needle.

Inoculation Method:

Fresh poultry manure was collected daily from laying hens. Manure was placed in plastic tubs (length 30 cm., height 12 cm. and width 23 cm.). Fly eggs were added to the manure by forming a shallow groove in the upper surface of the manure. The groove was gently closed after inoculation to prevent dehydration and loss of viability of the eggs (Figure 5).

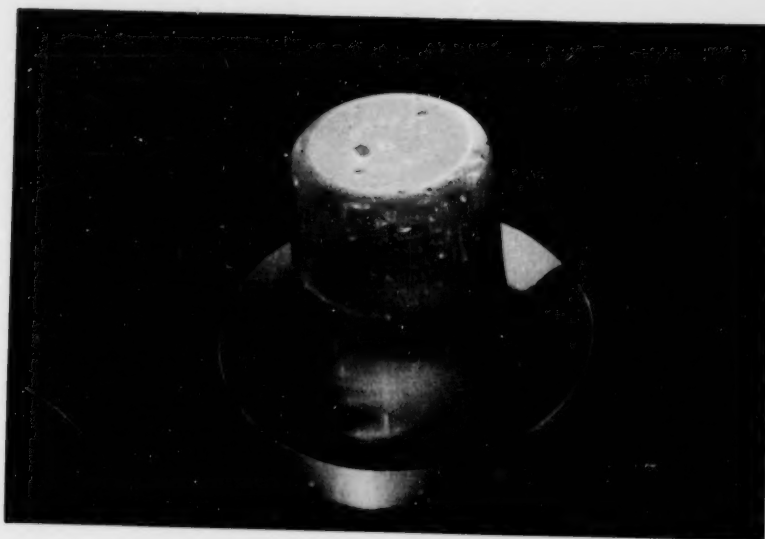


Figure 3. Device used for watering adult breeder flies.



Figure 4. Egg deposition tray with egg mass partly exposed.

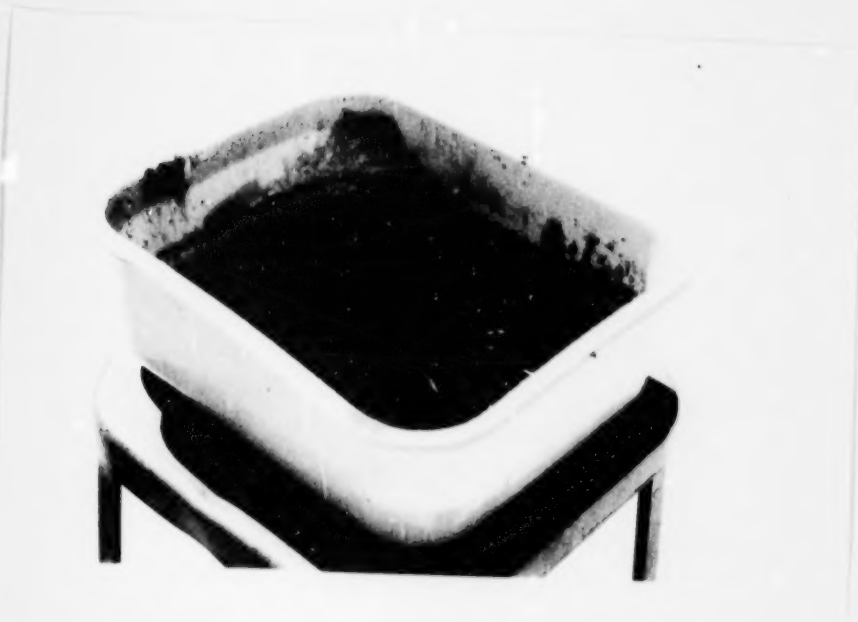


Figure 5. Tub of poultry manure showing the reduction in volume during catabolism by house fly larvae.

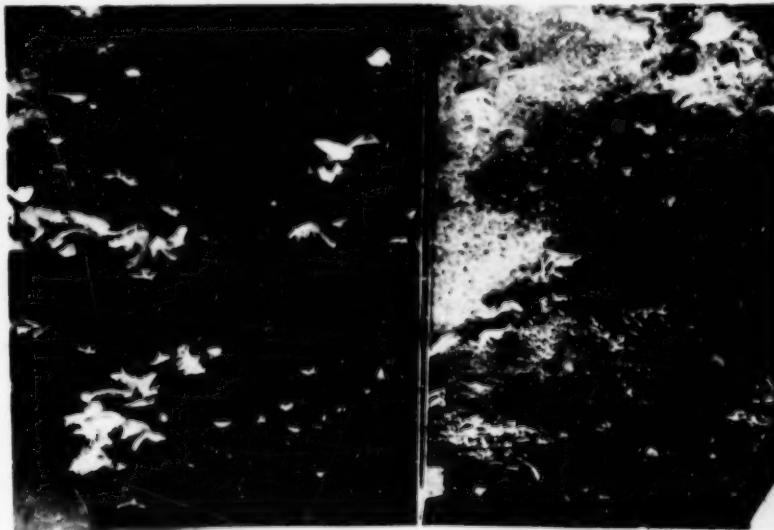


Figure 6. Modification of physical appearance of poultry manure by house fly larvae, left-control, right-inoculated with fly eggs.

Humidity and Temperature Studies:

The fly eggs were collected, separated from manure, weighed and inoculated in the fresh poultry manure daily. The inoculation rates varied from 2 to 5 g. of eggs per 4000 g. of poultry manure. These tubs were held under different temperatures (22 to 38° C.) and humidity conditions (19% to 80%) in a modified chick incubator. The tubs were weighed daily and weight loss was recorded. After six days 100 larvae were randomly picked from each tub. The larvae were put in petri dishes and held in a forced air drier at 65° C. overnight. The weight of the dry larvae was recorded. Pupae weight were obtained in the same fashion as larvae. Loss of moisture in the manure was recorded daily. The digested manure was carefully examined for changes in texture.

Hatchability Study:

A hatchability study of fly eggs in poultry manure was conducted by separating 200 fly eggs from the manure with the help of brush and blunt needle. These eggs were placed in a plastic bowl (diameter 9 cm. and height 8 cm.) which contained 500 g. fresh poultry manure. Manure with eggs was held at room temperature (approximately 27° C.). The number of larvae hatched in each bowl was counted after four days of development in the manure medium.

Harvesting of Pupae

The pupae were separated from the manure by flotation. The entire mass of manure which contained pupae was dumped into water. The manure particles sank and the bulk of the pupae floated. The pupae were removed by skimming the water with a piece of window screen. The pupae were spread in a metal tray (length 44 cm., height 5 cm. and width 26 cm.) and kept in a forced air drier at 65° C. overnight. Dry pupae were stored in metal buckets. The digested manure was also dried in the drier at 65° C.

Production of Fly Pupae Under Caged Layers:

Twenty-four White Leghorn adult hens were randomly divided into four groups containing six hens in each group. One group was kept as control, $\frac{1}{2}$ g. of fly eggs was inoculated into the droppings under each bird in the second group. One-day-old larvae and 400 g. manure were spread under the caged layers in the third group. Two-day-old larvae and 4000 g. manure were placed under the fourth group of layers. The texture of manure after digestion was carefully noted. The results were compared with that of the control. The experiment was repeated three times.

Feeding Trial No. I:

Forty-four day-old Single Comb White Leghorn cockerels were banded, weighed and randomly divided into eleven equal groups of

four chicks each. The chicks were maintained in electrically heated batteries with raised wire floors. Chicks received feed and water ad libitum and continuous lighting. The various rations fed to these chickens are presented in Table I. Each treatment was replicated twice except the sixth, where chicks were fed dead flies, corn, corn oil and pupae. Weight gain and feed consumption were determined weekly and mortality was recorded daily. The experiment was terminated after taking observations at four weeks of age.

Feeding Trial No. II:

Forty-eight day-old White Plymouth Rock broiler chicks were banded, weighed and randomly divided into eight equal groups. The chicks were maintained on litter in a brooder house with an electric brooder as a heat source. Diets fed to these chicks from day-old to seven weeks of age are presented in Table II. Each dietary treatment had four replications. Weight gain and feed consumption were recorded weekly and mortality was recorded daily. At seven weeks of age six chicks each from control and experimental groups were sacrificed for taste panel evaluation.

Feeding Trial No. III:

One hundred thirty-five day-old New Hampshire chicks and one hundred thirty-five day-old chicks of Indian River broiler strain were weighed, banded and randomly divided into twenty-seven equal

Table 1: Rations employed in studying feeding value of fly pupae to Single Comb White Leghorn chicks from one day to four weeks of age. (Feeding Trial #I).

Ingredients	Control	Values in percent unless noted			
		II	III	IV	V
Ground corn	31.00	31.00	31.00	31.00	31.00
Ground milo	22.60	22.60	22.90	22.60	22.90
Soybean oil meal (44%)	30.00	--	--	--	--
Ground fly pupae	--	30.00	30.00	--	--
Digested poultry manure	--	--	--	30.00	30.00
Fish meal (70%)	3.00	3.00	3.00	3.00	3.00
Meat and bone meal (50%)	5.00	5.00	5.00	5.00	5.00
Dehydrated alfalfa (17%)	2.50	2.50	2.50	2.50	2.50
Ground limestone	0.70	0.70	0.70	0.70	0.70
Dicalcium phosphate	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40	0.40
Corn oil	4.00	4.00	4.00	4.00	4.00
Vitamin mix*	0.25	0.25	--	0.25	--
Trace mineral mix**	0.05	0.05	--	0.05	--
Vitamin A (5280 IU/kg.)	No	No	Yes	No	Yes
Vitamin D ₃ (880 IU/kg.)	No	No	Yes	No	Yes
Total	100.00	100.00	100.00	100.00	100.00

* Vitamin mix supplies per kilogram of ration: Vitamin A 5280 IU; Vitamin D₃ 880 IU; Vitamin K 33.00 mg.; Riboflavin 4.40 mg; ³ Niacin 22.00 mg.; Pantothenic acid 4.40 mg.; and Choline (25%) 325.60 mg.

** Trace mineral mix supplies: Mn 50 ppm; Fe 50 ppm; Cu 5 ppm; Co 0.5 ppm and Zn 50 ppm.

Table II: Rations employed in studying feeding value of fly pupae to White Plymouth Rock chicks from one day to seven weeks of age. (Feeding Trial #II).

Ingredients	Values in percent unless noted	
	Control ration	Experimental ration
Ground corn	31.0	36.0
Ground milo	22.6	27.9
Soybean oil meal (44%)	30.0	--
Pupae	--	28.0
Fish meal (70%)	3.0	--
Meat and bone meal (50%)	5.0	--
Dehydrated alfalfa (17%)	2.5	2.5
Dicalcium Phosphate	0.5	0.6
Corn Oil	4.0	3.7
Salt	0.4	0.4
Ground limestone	0.7	0.9
Vitamin mix [*]	0.25	--
Trace mineral mix ^{**}	0.05	--
Methionine	0.05	--
Vitamin A	--	5280 IU/kg
Vitamin D ₃	--	880 IU/kg
Total	100.05	100.00

* Vitamin mix supplies per kilogram of ration: Vitamin A 5280 IU; Vitamin D₃ 880 IU; Vitamin K 33.00 mg; Riboflavin 4.40 mg; Niacin 22.00 mg; Pantothenic acid 4.40 mg; and Choline (25%) 325.60 mg.

** Trace mineral mix supplies: Mn 50 ppm; Fe 50 ppm; Cu 5 ppm; Co 0.5 ppm; and Zn 50 ppm.

groups of each breed. Each group was composed of five chicks. Each treatment had three replications in each breed. All chicks were maintained in electrically heated batteries with raised wire floors. The chicks received feed and water ad libitum and continuous lighting. Each group was fed one of the rations from one day to four weeks of age (Table III). Observations for gain in weight and feed consumption were taken weekly. Mortality was recorded daily.

Determination of Metabolizable Energy:

Broiler chickens were used in all experiments and were maintained in electrically heated, thermostatically controlled battery brooders with raised wire screen floors. They were reared to two weeks of age on the reference diet of Hill, et al. (1959). The chickens were then allotted to groups of ten chicks each on body weight, equalizing both mean body weight and weight distribution among the groups. Dried fly pupae and digested manure were incorporated in the experimental diets at a level of 30% at the expense of glucose. The experimental diet was fed to two duplicate lots for the period from two to four weeks of age. Feed and water were supplied ad libitum.

Excreta were collected at twenty-four hour intervals on four successive days during the last week of the experimental period. Chromium oxide was included at 0.3% of each diet as an index

substance. Excreta were dried in shallow pans immediately after each collection in a forced air drier at 70° C.

Analyses were performed on feed and excreta. Nitrogen was determined by macro-Kjeldahl procedure involving digestion with sulphuric acid in the presence of copper sulphate as catalyst. Combustible energy was determined by bomb calorimetry. Chromium oxide was determined spectrophotometrically following wet ashing according to the procedure described by Hill and Anderson (1958). Metabolizable energy was calculated according to the method of Hill, et al., (1959).

Amino Acid Analysis:

Three samples of 4.1 mg. ground dry pupae were weighed and transferred into standard 16 x 150 mm. Pyrex test tubes. One ml. of glass distilled 6N hydrochloric acid was added to each tube. Necking down of tubes was done with an oxygen gas flame. The tubes were kept in a dry ice-alcohol bath in a small Dewar flask until the acid sample mixture solidified. The tubes were evacuated to 50 micron Hg pressure using a high-vacuum mechanical pump. After evacuation the tubes were removed from the dry ice-alcohol bath and sealed while evacuated.

The sealed evacuated tubes were kept in an oven at $110^{\circ} \pm 1^{\circ}$ C. for 22 hours and then chilled in a refrigerator (4° C). Tops of the tubes were removed by making a deep scratch with a triangular file

and touching to the edge of the scratch the tip of a glass rod. The hydrochloric acid was removed by evaporating the sample to dryness. Five ml. of pH 2.2 buffer was pipetted into the tube containing the dried film of hydrolyzed sample. Analysis of the amino acid was conducted using a Beckman amino acid analyzer.

Chemical Analysis:

Analyses of pupae, feed and digested poultry manure were performed for different constituents (Tables 14, 16) according to the standard procedures listed in the Official Methods of Analysis of the Association of Official Agricultural Chemist (1965). Protein was determined by the macro-Kjeldahl method after digesting the material in concentrated sulphuric acid in the presence of copper sulphate as catalyst. A Soxhlet apparatus was used to determine the lipids. Extraction was performed with petroleum ether. Analysis for crude fiber was done on the defatted material according to Benedict's test. Minerals were analysed by atomic absorption spectrophotometry.

Statistical Methods:

Data collected in experiments were analysed statistically using a factorial design for the analysis of variance according to Snedecor and Cochran (1967). Significant differences between treatment means were determined by shortcut computation using range test (Kurtz, *et al.*, 1965) and Scheffee's method (Snedecor and Cochran, 1967).

CHAPTER IV

RESULTS AND DISCUSSION

Optimum yield of dry pupae was obtained with a combination of 3 g. fly eggs in 4000 g. of fresh poultry manure at 27° C. and a relative humidity of 41% (Table 4). At 22° C. and 60% relative humidity optimum production of pupae was obtained with a combination of 4 g. fly eggs and 4000 g. poultry manure.

As the quantity of eggs per g. of poultry manure was increased, the yield of pupae was depressed. Quite a few larvae were found dead in the tubs when five g. or more eggs were added to 4000 g. of poultry manure. This may be attributed to the inadequate food and space in the tub for larvae.

Moisture in the manure (Figure 7) was reduced from 78.5 to 55.0%. The physical consistency of the manure was changed from a sticky mass of material to a granulated consistency which greatly facilitated dehydration. Fresh poultry manure was difficult to dry because the dehydration sealed over surface prevented moisture loss from the center of the mass. The granulated structure dried readily due to physical structure, improved aeration and reduced initial moisture content. The residue was stabilized and was greatly reduced in odor but it retained a slight ammonia aroma along with earthy characteristics.

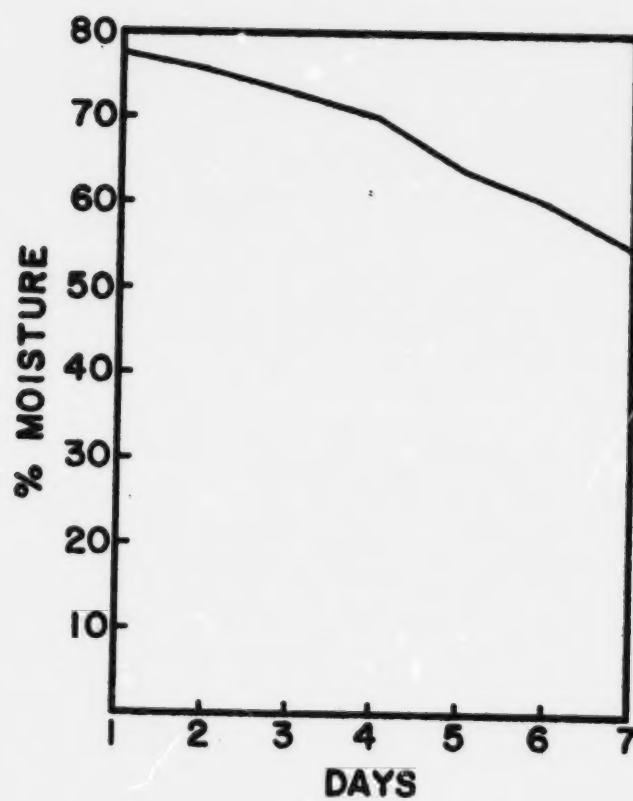


Figure 7. Moisture loss from fresh poultry manure during development of fly pupae.

Table IV: Yield of dry pupae from 4000 g. poultry manure at selected temperature and humidity conditions.

g. of fly eggs	Manure at harvest time		Temperature (° C.)	Relative	Weight of pupae (g.)
	Weight (g.)	%		humidity %	
2	2230	55.7	22	60	30
3	2150	53.7	22	60	45
4	2130	53.2	22	60	60
5	1980	49.5	22	60	39
2	2174	54.4	27 ¹	41	50
3	1949	48.7	27 ¹	41	76
4	2165	54.1	27 ¹	41	71

¹ Approximately eight days were required for pupae to develop from fly eggs.

As the environmental temperature declined from 27° C. the yield of pupae was also reduced (Table 4). At the environmental temperature of 20 - 21° C. the larvae in the manure appeared dormant. They were completely immobilized when the temperature was 17° C. or below.

The environmental humidity had a profound effect on the yield of pupae (Table 5). When the relative humidity was increased from 38 to 70%, the yield of pupae at temperatures of 34 to 38° C. was significantly increased ($P < 0.01$). Most of the larvae were found dead at 19% relative humidity when the temperature varied from 32 to 38° C. (Table 6). Larvae weight under these conditions was also decreased. The manure at the above conditions (relative humidity 19%, temperature 32 to 38° C.) could not be digested sufficiently by the young larvae.

Approximately eight days were required for the fly eggs to be converted to pupae in fresh poultry manure at 27° C. and 41% relative humidity.

Moisture in Manure:

When the moisture content of the manure was above 80%, the larvae left the manure mass and climbed out of the plastic containers. In this process a large number of larvae escaped from the container. The plastic container could not be climbed directly. It was first roughened with dried fecal matter, so each successive attempt to

Table V: Influence of environmental temperature and relative humidity on the yield of fly pupae (4000 g. poultry manure inoculated with 4 g. fly eggs).

Sample No.	Manure at harvest time Weight (g.)	Temperature %	Temperature (° C.)	Relative humidity %	Weight of dry pupae (g.)
1	1156	28.9	34	38	19.5
2	1282	32.0	36	38	18.0
3	1173	29.3	38	38	10.0
4	1266	31.6	34	44	25.0
5	1350	33.7	36	44	23.0
6	1396	34.9	38	44	20.0
7	1640	41.0	34	58	28.0
8	1643	41.0	36	58	26.0
9	1636	40.9	38	58	25.0
10	2400	60.0	34	70	40.0**
11	2350	58.7	36	70	38.0**
12	2340	58.5	38	70	42.0**

** Significant at 0.01 level of probability.

Table VI: Effect of environmental temperature and relative humidity (19%) on weight of larvae.

Sample No.	Manure/eggs (g.)	Temperature (° C.)	Weight of 100 dry larvae (g.)
1	4000/2	32	0.175**
2	4000/3	32	0.125
3	4000/2	35	0.150**
4	4000/3	35	0.080
5	4000/2	38	0.130**
6	4000/3	38	0.075

** Significant at 0.01 level of probability.

climb out was rewarded with a further advance up the wall of the container. The scaling of the container wall was not an organized action of the larvae but occurred incidentally to the gross activity of the larvae attempting to escape the unsuitable environment of the manure mass.

Studies on random samples of fly eggs indicated that the average fly eggs weighed 0.049 mg. Hatchability of the fly eggs in fresh poultry manure varied from 50.5 to 87.0% (Appendix Table 10). This variation may have been caused by age of the eggs and by crushing or puncturing the eggs during handling and counting.

Development of all the flies was not uniform as some larvae pupated earlier than others. Different ages of eggs may have contributed to some of the variation in maturation. As the fly eggs hatched, the larvae moved into the manure mass to feed. Very young larvae tended to feed in groups near the site of egg inoculation. As they became larger, they burrowed throughout the manure mass.

The combination of 2 g. eggs per 4000 g. of fresh poultry manure produced significantly heavier larvae under different environmental temperature and relative humidity conditions (Tables 7, 8 and 9). This combination was not suitable for the maximum yield of pupae (Table 4). Even though the combination gave larger larvae the number of larvae was reduced so that maximum yield of pupae could not be obtained with the above combination and a greater amount of manure residue remained.

Table VII: Effect of environmental temperature and relative humidity (40%) on weight of larvae.

Sample No.	Manure/eggs (g.)	Temperature (° C.)	Weight of 100 dry larvae (g.)
1	4000/2	32	0.420 **
2	4000/3	32	0.385
3	4000/2	35	2.395 **
4	4000/3	35	0.280
5	4000/2	38	0.400 **
6	4000/3	38	0.260

** Significant at 0.01 level of probability.

Table VIII: Effect of environmental temperature and relative humidity (59%) on weight of larvae.

Sample No.	Manure/eggs (g.)	Temperature (° C.)	Weight of 100 dry larvae (g.)
1	4000/2	32	0.680**
2	4000/3	32	0.610
3	4000/4	32	0.595
4	4000/2	35	0.700**
5	4000/3	35	0.690
6	4000/4	35	0.580
7	4000/2	38	0.685**
8	4000/3	38	0.615
9	4000/4	38	0.525

** Significant at 0.01 level of probability.

Table IX: Effect of environmental temperature and relative humidity (80%) on weight of larvae.

Sample No.	Manure/eggs (g.)	Temperature (° C.)	Weight of 100 dry larvae (g.)
1	4000/2	32 ¹	0.600 **
2	4000/3	32 ¹	0.565
3	4000/4	32 ¹	0.455
4	4000/2	35	0.650 **
5	4000/3	35	0.575
6	4000/4	35	0.460
7	4000/2	38	0.570 **
8	4000/3	38	0.570
9	4000/4	38	0.450

** Significant at 0.01 level of probability.

¹ Observed fungal development in manure.

Table X: Digestion of manure under caged birds.

g. of eggs or larvae/bird	No. of hens	No. of days	Dry weight of manure including larvae and pupae (g.) ¹	% moisture at the time of harvest
$\frac{1}{2}$ g. egg/bird	6	12	1457	65.0
Day old larvae, 4000 g. manure	6	10	1785	67.5
Two day old larvae, 4000 g. manure	6	9	1522	50.0
Control	6	--	2098	80.0

¹ Not subjected to analysis of variance.

Digestion of Manure Under Caged Birds:

Fly eggs and/or larvae can be used to digest the manure under the caged birds (Table 10). Digested manure was greatly reduced in odor but it retained a slight ammonia aroma along with an earthy characteristic. The manure was loose and crumbly in texture in comparison to the control sample (Figure 6). The moisture content of the digested manure varied from 50.0 to 67.5%, whereas the moisture content of manure in the control group was 80%. It was assumed in this experiment that all the hens would deposit comparable amounts of fecal material. Data indicated that manure mass in treated groups was reduced more than the control. Eleven to twelve days were required for the larvae to develop to the pupal stage. Low temperature (20° C.) in the poultry house was found to be a contributing factor for slow larval development.

Feeding Trial No. 1:

Chicks fed a diet containing pupae (Table 11) showed no significant difference in weight gain when compared with chicks receiving the control diet during the experimental period from one day to four weeks of age. Chicks receiving the pupae diet (no trace mineral or B vitamin supplement, only vitamins A and D₃ added) showed better and more uniform growth, when compared to all other groups throughout the experimental period (Figure 8). Chicks fed a diet containing digested manure had reduced weight gain, but this

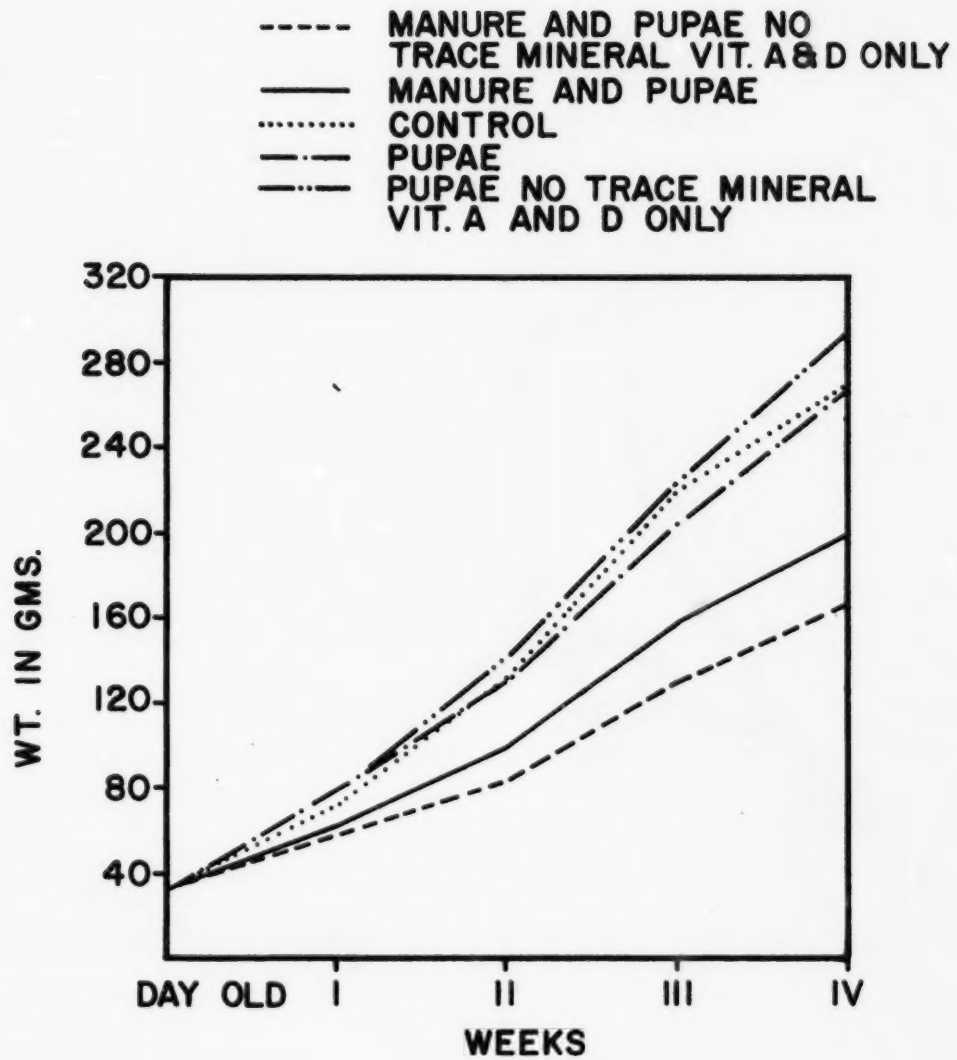


Figure 8. Body weight (g) of Single Comb White Leghorn chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial #1)

Table XI: Average body weight gain (g) of Single Comb White Leghorn chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial #I). ^{ns}

Treatments	1st week	2nd week	3rd week	4th week	Total
I. Control	40	59	88	50	237
II. Pupae	44	53	74	65	236
III. Pupae (no trace mineral; vitamins A and D ₃ added)	45	63	83	69	260
IV. Digested manure	28	36	60	42	166
V. Digested manure (no trace mineral; vitamins A and D ₃ added)	25	25	47	38	135
VI. Flies, corn, corn oil and pupae	32	50	58	53	193

^{ns} Values are not significantly different at 0.05 level of probability.

Table XII: Feed conversion (g. of feed/g. of gain) of Single Comb White Leghorn chicks receiving various dietary treatment containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial #I).

Treatments	1st week	2nd week	3rd week	4th week	Average
I. Control	2.00	1.75	1.75	3.95	2.36
II. Pupae	1.60	1.90	2.05	2.80	2.09
III. Pupae (no trace mineral; vitamins A and D ₃ added)	1.40	1.70	2.00	2.55	1.91
IV. Digested manure	2.90	2.90	2.80	4.60	3.30*
V. Digested manure (no trace mineral; vitamins A and D ₃ added)	3.20	4.20	3.25	4.15	3.70*

* Significant at 0.05 level of probability.

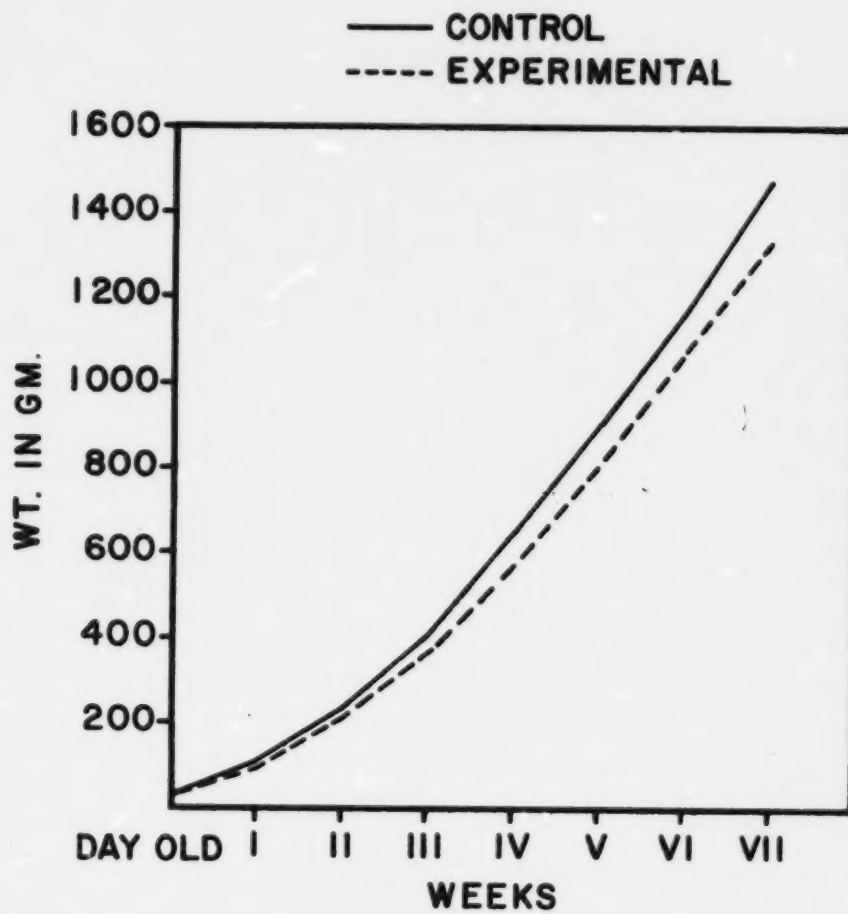


Figure 9. Body weight (g) of White Plymouth Rock chicks . receiving control and pupae rations from one day to seven weeks of age. (Feeding Trial #II)

difference was not significant ($P < 0.05$). Chicks fed only dead flies, corn, corn oil and pupae gained more weight than the chicks fed the diet containing manure. These results agreed well with those of Calvert, et al. (1969) who reported improved growth of chicks when pupae replaced soybean oil meal in the diet.

No significant difference in feed conversion was obtained between the control chicks and chicks receiving pupae in their diet (Table 12). Feed conversion of the chicks fed digested manure was significantly lower ($P < 0.05$) when compared to the controls. The lower protein and higher fiber content of the digested manure (Appendix Table 3) may account for this difference. Total feed consumption of the chicks fed pupae was 513 - 518 g. whereas the control chicks consumed 523 g. of feed (Appendix Table I). Mortality in all groups was less than 1%.

Feeding Trial No. II:

Chicks fed pupae as the only protein supplement (Table 13) showed no significant difference in weight gain when compared with chicks fed the control diet during the seven weeks of the experimental period. However, the gain in weight of chicks fed pupae was lower than control chicks (Figure 9). There was no significant difference in feed consumption and feed conversion between the control and experimental groups. Chicks on the pupae diet consumed less feed than the chicks on the control diet. Average feed conversion at the

end of the experimental period for the control and experimental groups was 2.17 and 2.06, respectively. Mortality in both groups was less than one percent.

No difference in carcass quality and taste was detected by an informal taste panel between the birds fed control and experimental diets.

Metabolizable Energy:

Metabolizable energy of the fly pupae was determined to be 2528 Kcal. per kg., which is higher than soyabean oil meal and slightly lower than fish meal. The metabolizable energy of the digested manure was determined to be 580 Kcal. per kg.

Chemical Analysis:

Proximate analysis of pupae (Table 14) indicated that they contained 61.4% protein and 9.3% fat. The amino acid composition (Table 15) indicated that protein quality was similar to that of meat and bone meal or fish meal, and even better than soybean oil meal. Thus pupae contain a sufficient quality and quantity of protein and other nutrients (as indicated by feeding trials) to be a suitable substitute for soybean oil meal and fish meal in the diet of baby chicks. Chemical analysis indicated fly pupae should be a good source of trace minerals. The data on protein, amino acid and fat analysis differ with that of Calvert, et al. (1969). This could have

Table XIII: Average weight gain (g), average feed consumption (g) and feed conversion (g. of feed/g. of gain) of White Plymouth Rock chicks receiving control and pupae ration from one day to seven weeks of age. (Feeding Trial #II)^{ns}

Weeks	Control ration			Pupae ration		
	Weight gain	Feed consumption	Feed conversion	Weight gain	Feed consumption	Feed conversion
1	70	171	2.4	63	166	2.6
2	129	254	1.9	111	230	2.0
3	181	316	1.7	160	241	1.5
4	237	444	1.9	205	368	1.8
5	256	588	2.3	239	524	2.2
6	267	619	2.3	258	528	2.0
7	289	709	2.4	252	590	2.3
Total	1429	3101	-	1288	2647	-
Average	-	-	2.17	-	-	2.06

^{ns} Values are not significantly different at 0.05 level of probability.

Table XIV: Proximate analysis of dried fly pupae produced in fresh poultry manure.

Constituents	%
Protein	61.4
Fat	9.3
Ash	11.9
N. F. E. and Fiber	18.4

Mineral content of dried fly pupae		
P	1.43%	
Ca	0.93%	
K	0.88%	
Na	0.56%	
Mn	370	ppm
Cu	34	ppm
Zn	275	ppm
Fe	465	ppm

Chemical analysis conducted by Triple "S" Laboratories,
Loveland, Colorado.

Table XV: Amino acid analysis of fly pupae.

Amino acid	Values in percent
Arginine	3.7
Glycine	2.4
Histidine	2.0
Isoleucine	2.4
Leucine	3.4
Lysine	3.8
Methionine	1.6
Phenylalanine	3.0
Threonine	2.1
Valine	2.7
Glutamic acid	7.2
Alanine	2.5
Tyrosine	3.8
Proline	2.3
Serine	1.9
Aspartic acid	5.3
Ammonia	11.1

Table XVI: Chemical analysis of poultry manure on a dry matter basis before and after digestion with fly larvae.

Constituents	Fresh manure	Digested manure
Values as percent unless noted		
Nitrogen	7.5	2.6
Protein	--	17.62
Crude fat	2.64	1.64
Crude fiber	18.12	19.08
Ash	22.73	30.13
Nirtogen free extract	42.05	34.59
Calcium	5.119	6.976
Phosphorus	3.421	1.876
Magnesium	0.304	0.626
Potassium	1.679	3.132
Sodium	0.239	0.756
Sulfur	--	0.147
Manganese ppm	276.0	432.0
Copper ppm	68.0	59.4
Cobalt ppm	2.8	2.8
Zinc ppm	376.0	544.9
Iron ppm	560.0	544.0
Molybdenum ppm	2.8	2.2

Chemical analysis conducted by Triple "S" Laboratories, Loveland, Colorado.

been attributed to the difference in the diet of the fly larvae and digestion procedure of samples during analysis.

Chemical Analysis of the Poultry Manure After Digestion:

The analysis of poultry manure (Table 16) after digestion indicated that a part of nitrogen was converted to proteins except for some that was lost as ammonia during the digestion. All the constituents except magnesium, potassium, sodium, cobalt and molybdenum were relatively elevated in comparison to fresh poultry manure. The difference could be due partially to selective absorption of minerals by larvae.

Feeding Trial No. III:

Chicks fed pupae, in lieu of the fish meal and meat and bone meal of the control ration (Tables 17 and 18) showed no significant ($P < 0.05$) difference in weight gain when compared with chicks fed control diet during the experimental period of four weeks. The commercial broiler strain when fed digested poultry manure at different levels had less gain in weight than control groups, but this difference was not significant ($P < 0.05$). New Hampshire chicks, fed pupae, separated by drying and screening, gained more weight than controlled chicks. The chicks fed digested poultry manure at different levels also gained more weight than controls. These differences were not significant ($P < 0.05$). Chicks on a pupae ration

that was deficient in protein gained more weight than the chicks on the control diet which was also deficient in protein. The data gives an indication that fly pupae has a quality of protein comparable to the fish meal and meat and bone meal of the control diet to support the growth of the baby chicks.

No significant difference ($P < 0.05$) in the feed conversion was obtained between the control and chicks fed diets which contained pupae and digested manure (Tables 19 and 20). The feed conversion of chicks fed diet containing pupae (26.55% protein) was comparable to chicks fed controlled diet (25.26% protein). Similarly the feed conversion of the chicks fed diet containing pupae (16.86% protein) was also comparable to chicks fed controlled diet (17.38% protein). Feed consumption was greater in the chicks fed digested manure than any other groups in the experiment (Appendix Tables 6 and 7). Mortality in all groups was less than one percent.

Table XVII: Average gain (g.) of Indian River chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial No. III). ^{ns}

Treatments	1st week	2nd week	3rd week	4th week	Total
Control (25.26% Protein)	46.2	113.5	159.3	187.5	506.5
Control (17.38% Protein)	36.2	74.7	126.0	154.4	391.3
Pupae (flotation)	44.5	105.3	140.0	194.9	484.7
Pupae (16.86% Protein)	35.7	88.7	128.1	156.0	408.5
Pupae (by drying and screening)	48.8	105.8	152.4	196.3	503.3
Poultry manure 5%	44.5	89.3	128.0	193.8	455.6
Poultry manure 10%	45.7	99.7	140.5	186.6	472.5
Screened manure	40.2	103.3	106.5	212.5	462.5
Control diet 80 parts; manure 20 parts	47.4	148.3	108.5	173.9	478.1

^{ns} Means are not significantly different at 0.05 level of probability.

Table XVIII: Average gain in weight (g.) of New Hampshire chicks receiving various dietary treatments containing fly pupae and digested manure from one day, to four weeks of age. (Feeding Trial No. III) ^{ns}

Treatments	1st week	2nd week	3rd week	4th week	Total
Control (25.26% Protein)	34.5	68.3	91.7	105.4	299.9
Control (17.38% Protein)	30.3	55.9	75.5	91.0	252.7
Pupae (flotation)	30.7	68.9	68.7	124.3	292.6
Pupae (16.86% Protein)	36.4	62.5	76.6	94.9	270.4
Pupae (by drying and screening)	42.3	78.3	95.7	133.0	349.3
Poultry manure 5%	34.3	84.4	107.4	122.6	348.7
Poultry manure 10%	41.7	78.2	100.6	136.3	356.8
Screened manure	39.0	76.8	110.5	130.7	357.0
Control diet 80 parts; screened manure 20 parts	36.3	68.0	93.7	108.4	306.4

^{ns} Means are not significantly different at 0.05 level of probability.

Table XIX: Feed conversion of Indian River chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial No.III)^{ns}

Treatments	(g. of feed/g. of gain)				Average
	1st week	2nd week	3rd week	4th week	
Control (25.26% Protein)	1.23	1.44	1.65	1.67	1.49
Control (17.38% Protein)	1.49	1.94	1.99	2.00	1.85
Pupae (flotation)	1.33	1.49	1.82	1.68	1.58
Pupae (16.86% Protein)	1.55	1.83	2.02	1.98	1.84
Pupae (by drying and screening)	1.22	1.42	1.70	1.63	1.49
Poultry manure 5%	1.25	1.58	1.79	1.68	1.57
Poultry manure 10%	1.24	1.71	2.17	1.83	1.74
Screened manure	1.42	1.40	2.34	1.76	1.73
Control diet 80 parts; manure 20 parts	1.30	1.56	3.10	2.00	1.99

^{ns} Means are not significantly different at 0.05 level of probability.

Table XX: Feed conversion of New Hampshire chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial No. III) ^{ns}

Treatments	(g. of feed/g. of gain)				Average
	1st week	2nd week	3rd week	4th week	
Control (25.26% Protein)	1.85	1.69	2.28	2.16	1.99
Control (17.38% Protein)	2.04	1.78	2.73	2.40	2.24
Pupae (flotation)	1.84	1.54	2.66	2.00	2.01
Pupae (16.86% Protein)	1.98	2.06	2.78	2.50	2.33
Pupae (by drying and screening)	1.63	1.55	2.19	1.97	1.83
Poultry manure 5%	1.63	1.43	1.96	1.95	1.74
Poultry manure 10%	1.71	1.65	2.19	2.04	1.89
Screened manure	1.68	1.49	2.05	1.96	1.79
Control diet 80 parts; manure 20 parts	1.81	1.91	2.47	2.35	2.13

^{ns} Means are not significantly different at 0.05 level of probability.

SUMMARY

Environmental temperature of 27°C was found best for the growth of larvae in poultry manure when the environmental relative humidity was approximately 41%. At these environmental conditions a combination of 3 g. fly eggs in 4000 g. fresh poultry manure gave optimum yield of dry fly pupae. Best growth of larvae was observed when the range of moisture in the manure was 70 to 75%. The developing larvae abandoned the manure mass when its moisture content reached 80% or above.

Moisture in the manure was reduced from 78.5% to 55.0%. Relative humidity affected the growth of larvae. When the relative humidity was increased from 38% to 70% the yield of pupae at 34 to 38°C was increased ($P < 0.01$). Average fly eggs weighed 0.049 mg. and their hatchability in fresh poultry manure varied from 50.5% to 87.0%. Fungal development was observed in the manure when the temperature was held at 32°C and relative humidity increased to 80%.

In comparison to fresh poultry manure the manure residue had less odor, contained less moisture and was granular in texture. The fly larvae developed well under the caged layers, reducing freshly voided manure to granular material within a few hours.

White Leghorn chicks fed the diet containing fly pupae had slightly better feed conversion with weight gains comparable to the control but the difference was not significant ($P < 0.05$). Replacement of all soybean meal with manure residue failed to support growth of chicks at the level of comparable controls. The feed conversion of these chicks was significantly less desirable ($P < 0.05$). White Plymouth Rock broiler chicks fed pupae as the only protein supplement showed no significant ($P < 0.05$) difference with respect to weight gain and feed conversion when compared to control chicks. Indian River and New Hampshire chicks fed pupae and digested poultry manure (5 to 10%) showed no significant ($P < 0.05$) difference in weight gain and feed conversion when compared with control chicks.

The results of these evaluations indicated that fly pupae have potential as a protein supplement in chick starter and broiler diets. The amino acid composition indicated that protein quality was similar to that of meat and bone meal or fish meal, and superior to soybean oil meal.

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APPENDIX

Appendix Table I

Average feed consumption (g.) of Single Comb White Leghorn chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial No. 1).

	1st week	2nd week	3rd week	4th week	Total
I Control (chick starter)	82	104	156	181	523
II Pupae	72	101	152	188	513
III Pupae (no trace mineral; vitamins A and D ₃ added)	59	109	168	182	518
IV Digested manure	84	103	167	194	548
V Digested manure (no trace mineral; vitamins A and D ₃ added)	82	105	153	177	517

Appendix Table II

Calculated analysis of the diets employed in studying the feed value of fly pupae and digested poultry manure to Single Comb White Leghorn chicks. (Feeding Trial No. 1).¹

Constituents		Diet No. I	Diet No. II & III	Diet No. IV & V
Protein	%	23.0	27.26	14.36
Fat	%	6.4	9.57	7.17
Fiber	%	3.8	2.59	7.69
Calcium	%	1.0	0.96	3.03
Phosphorus	%	.77	0.97	1.12
Lysine	%	1.4	1.94	0.44
Methionine and cystine	%	0.8	1.39	0.37
Metabolizable energy		2963 Cal. /kg.	3137 Cal. /kg.	2767 Cal. /kg.

¹ Amino acid content of digested poultry manure is not known, therefore calculated analysis does not include the amino acid present in the manure.

Appendix Table III

Chemical analysis for diets employed in studying the feed value of fly pupae and digested poultry manure to Single Comb White Leghorn chicks. (Feeding Trial No. 1).

Constituents	Diet #1	Diet #2	Diet #3	Diet #4	Diet #5
Protein %	24.03	27.33	27.07	16.32	16.45
Crude Fat %	8.72	12.54	13.03	7.72	8.18
Crude Fiber %	5.08	5.38	5.31	6.79	8.28
Ash %	7.36	8.19	8.16	13.40	13.53
Nitrogen Free Extract %	55.14	47.27	47.14	56.73	54.65
Calcium %	1.240	1.529	1.480	3.258	3.207
Phosphorus %	0.818	1.128	0.998	1.514	1.548
Magnesium %	0.184	0.243	0.197	0.309	0.303
Potassium %	1.176	0.871	0.728	1.640	1.256
Sodium %	0.344	0.551	0.472	0.486	0.519
Sulfur %	0.109	0.095	0.119	0.207	0.166
Manganese ppm	79.6	132.3	101.2	223.6	136.7
Copper ppm	18.0	32.4	26.0	39.6	32.4
Cobalt ppm	0.9	1.6	1.0	1.6	1.4
Zinc ppm	112.8	156.4	115.1	261.9	184.9
Iron ppm	173.0	287.1	222.2	370.1	415.7
Molybdenum ppm	1.1	1.9	3.0	3.0	3.2

Chemical analysis conducted by Triple "S" Laboratories, Loveland, Colorado.

Appendix Table IV

Calculated analysis for the diets employed in studying feed value of fly pupae to White Plymouth Rock chicks. (Feeding Trial No. 2).

Constituents		Basal Diet	Experimental Diet
Protein	%	23.00	23.00
Fat	%	6.40	8.50
Fiber	%	3.80	2.10
Calcium	%	1.00	1.10
Phosphorus	%	0.77	0.71
Lysine	%	1.40	1.60
Methionine and Cystine	%	0.80	1.05
Metabolizable energy		2963 Cal. /kg.	2992 Cal. /kg.
Calorie:protein ratio		58:1	59:1

Appendix Table V

Chemical analysis of the diets employed in studying the feed value of fly pupae to White Plymouth Rock chicks. (Feeding Trial No. 2).

Constituents	Control diet	Experimental diet (pupae)
Values as percent unless noted		
Protein	26.41	24.51
Crude fat	8.44	10.26
Crude fiber	3.72	5.93
Ash	6.47	5.56
Nitrogen free extract	51.41	54.66
Calcium	1.282	1.318
Phosphorus	0.707	0.726
Magnesium	0.128	0.170
Potassium	0.854	0.680
Sodium	0.162	0.187
Sulfur	0.075	0.091
Manganese ppm	85.4	119.0
Copper ppm	21.3	29.7
Cobalt ppm	0.6	0.2
Zinc ppm	196.5	229.6
Iron ppm	162.3	178.6
Molybdenum ppm	1.7	1.7

Chemical analysis conducted by Triple "S" Laboratories, Loveland, Colorado.

Appendix Table VI

Average feed consumption (g.) of Indian River chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial No. 3).

Treatments	1st week	2nd week	3rd week	4th week	Total
Control (25.26% protein)	57.0	163.7	262.4	365.4	848.5
Control (17.38% protein)	54.0	142.7	250.6	339.3	786.6
Pupae (flotation)	59.3	157.5	253.1	346.4	816.3
Pupae (16.86% protein)	58.7	162.4	258.5	329.6	809.2
Pupae (by drying and screening)	59.7	146.6	258.3	365.7	830.3
Poultry manure 5%	55.5	154.8	247.7	350.1	808.1
Poultry manure 10%	56.8	166.8	303.4	381.2	908.2
Screened manure	57.1	144.6	227.8	383.0	812.5
Control diet 80 parts; manure 20 parts	61.8	176.5	315.0	394.4	947.7

Appendix Table VII

Average feed consumption (g.) of New Hampshire chicks receiving various dietary treatments containing fly pupae and digested manure from one day to four weeks of age. (Feeding Trial No. 3)

Treatments	1st week	2nd week	3rd week	4th week	Total
Control (25.26% protein)	63.3	144.4	208.6	260.7	677.0
Control (17.38% protein)	61.4	99.5	203.2	239.7	603.8
Pupae (flotation)	55.3	105.2	177.4	259.2	597.1
Pupae (16.86% protein)	72.2	127.9	212.1	255.7	667.9
Pupae (by drying and screening)	69.0	121.9	207.6	292.3	690.8
Poultry manure 5%	62.0	120.8	211.0	287.5	681.3
Poultry manure 10%	71.7	130.1	218.0	279.3	699.1
Screened manure	65.8	114.8	227.6	293.5	701.7
Control diet 80 parts; manure 20 parts	65.0	130.1	232.7	293.5	721.3

Appendix Table VIII

Calculated analysis of the diets employed for the study of fly pupae and digested poultry manure to Indian River and New Hampshire chicks. (Feeding Trial No. 3)

Constituents		1	2	3	4	6	7
Protein	%	23.00	14.70	22.61	15.00	23.64	24.00
Fat	%	6.40	6.67	6.70	6.56	6.89	6.82
Fiber	%	3.80	2.75	3.62	2.58	4.09	5.56
Calcium	%	1.00	.99	.55	.61	1.28	1.63
Phosphorous	%	.77	.62	.59	.49	.82	.91
Lysine	%	1.40	.80	1.33	.91	1.22	1.20
Methionine cystine	%	.80	.68	.87	.64	.80	.78
Metabolizable energy							
Cal. /kg.		2963	3113	3027	3226	2961	2873
C:P. ratio		58:1	102:1	60:1	100:1	57:1	54:1

¹ Since the chemical analysis of manure fraction and pupae (separated from machine) was not known, therefore the analysis of diet No. 5, 8 and 9 could not be calculated.

Appendix Table IX

Chemical analysis of the diets employed in studying the feeding value of fly pupae and digested poultry manure to Indian River and New Hampshire chicks. (Feeding Trial No. 3).

Constituents	I	II	III	IV	V	VI	VII	VIII	IX
Values in percent unless noted									
Protein	25.26	17.38	26.55	16.86	26.20	28.35	27.00	26.82	25.71
Crude fat	7.38	6.84	8.32	7.10	7.05	7.88	7.64	7.44	6.64
Crude fiber	4.29	2.69	5.05	3.42	6.04	7.43	6.55	5.83	7.17
Ash	6.49	5.61	6.10	4.21	6.16	8.28	9.81	9.39	11.88
Nitrogen free extract	57.40	68.11	54.71	68.95	55.38	48.85	49.83	51.30	49.69
Calcium	1.656	1.546	1.233	0.953	1.059	1.562	2.083	1.993	2.614
Phosphorus	0.918	0.804	0.536	0.488	0.557	0.888	0.996	0.980	1.317
Magnesium	0.183	0.128	0.176	0.129	0.167	0.156	0.190	0.182	0.226
Potassium	0.958	0.601	0.969	0.519	0.971	0.867	1.128	0.953	1.113
Sodium	0.156	0.146	0.158	0.147	0.167	0.147	0.182	0.164	0.209
Sulfur	0.071	0.070	0.083	0.076	0.125	0.161	0.248	0.224	0.182
Manganese ppm	78.4	73.0	132.1	90.9	127.9	91.1	130.1	104.0	152.5
Copper ppm	13.0	4.2	17.6	4.3	26.4	17.3	26.9	21.6	39.2
Cobalt ppm	0.8	0.6	0.6	0.2	0.4	1.0	1.3	1.1	1.7
Zinc ppm	217.9	180.3	229.1	199.3	247.1	234.3	286.4	242.6	287.6
Iron ppm	152.5	154.6	180.6	151.6	203.0	195.2	260.3	234.0	326.8
Molybdenum ppm	1.7	1.2	1.4	1.3	0.2	2.6	2.1	1.7	2.1

Appendix Table X

Hatchability study of fly eggs in fresh poultry manure.

Sample No.	No. of eggs kept ¹	No. of larvae ²	% Hatchability ³
1	200	150	75.0
2	200	167	83.5
3	200	135	67.5
4	200	121	60.5
5	200	101	50.5
6	200	124	62.0
7	200	174	87.0

¹ Average weight of fly egg .049 mg.

² Average length of larvae 4 mm.

³ Not subjected to analysis of variance.